

Nitrogen accumulation in eastern oysters (*Crassostrea virginica*) varies significantly across the Delaware
Inland Bays

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Abstract

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Bivalve aquaculture is increasingly being considered as a viable bioremediation strategy in eutrophied estuaries. Bivalves provide a variety of ecosystem services, including water filtration and nutrient removal from the water column. Filtration rates vary according to species and location-specific hydrological conditions. To explore the potential benefits of using eastern oyster (*Crassostrea virginica*) aquaculture as part of a nutrient trading plan, I examined nitrogen (N) accumulation across the Delaware Inland Bays, a series of small estuaries on the Mid-Atlantic coast. Two different size classes of oysters were deployed in multiple locations to monitor N content across space and N accumulation rate according to size. I found that N content varied with location but not with size class. N content in oyster tissue (% DW) was similar to previous findings for *C. virginica*, but content in shell (% DW) was up to an order of magnitude greater. My results confirm that N accumulation varies depending on location and suggests that N content within the shell may be higher than previously reported. My attempts to quantify the nutrient bioassimilation services that oyster aquaculture provides could be used to help inform future nutrient management plans in the Delaware Inland Bays.

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Abstract

Bivalve aquaculture is increasingly being considered as a viable bioremediation strategy in eutrophied estuaries. Bivalves provide a variety of ecosystem services, including water filtration and nutrient removal from the water column. Filtration rates vary according to species and location-specific hydrological conditions. To explore the potential benefits of using eastern oyster (*Crassostrea virginica*) aquaculture as part of a nutrient trading plan, I examined nitrogen (N) accumulation across the Delaware Inland Bays, a series of small estuaries on the Mid-Atlantic coast. Two different size classes of oysters were deployed in multiple locations to monitor N content across space and N accumulation rate according to size. I found that N content varied with location but not with size class. N content in oyster tissue (% DW) was similar to previous findings for *C. virginica*, but content in shell (% DW) was up to an order of magnitude greater. My results confirm that N accumulation varies depending on location and suggests that N content within the shell may be higher than previously reported. My attempts to quantify the nutrient bioassimilation services that oyster aquaculture provides could be used to help inform future nutrient management plans in the Delaware Inland Bays.

Introduction

Anthropogenic nutrient input to coastal waters, a primary driver of eutrophication, has been increasing since the 1960s, and by the year 2050, nitrogen and phosphorus loading into coastal systems is expected to double globally compared to today's levels (Diaz et al. 2013). Bivalve aquaculture has been considered a viable strategy for addressing eutrophication through bioremediation for some decades (Officer et al. 1982), yet nutrient trading programs are a relatively new policy tool (Golen 2007). Bivalves are ecosystem engineers that provide a variety of services including enhanced water quality and nutrient removal and cycling from the water column through suspension-feeding (Shumway et al. 2003, Coen et al. 2007, Alleway et al. 2019). During feeding, bivalves filter phytoplankton from the water column and assimilate those nutrients into their tissue and shell as they grow (Newell 2002). Bivalves typically remove phytoplankton cells from seawater faster than cells can be replaced through growth, giving them substantial capacity to increase clarity of the water column via phytoplankton consumption (Officer et al. 1982). The eastern oyster, *Crassostrea virginica* (Gmelin, 1791), is an especially attractive candidate for biofiltration because it has a high feeding, or clearance, rate compared to other cultured bivalves, and it can maintain its feeding rate in the presence of high volumes of particulate matter (Newell 2004, Dame 2012).

The presence of oysters also influences N cycling through biodeposition and other biogeochemical pathways including denitrification (Newell et al. 2002). N is returned to the water column through biodeposition in the form of feces or pseudofeces after consumption and can be either buried (removed) or resuspended (recycled) thereafter (Smyth et al. 2013). Several biogeochemical processes, including microbially-mediated denitrification, can occur at the sediment level (Smyth et al. 2018). These processes release N back into the water column as the gaseous form, N_2 , which is eventually outgassed into the atmosphere, thereby constituting net removal from the water column (Newell et al. 2002). Estuary-specific conditions, including salinity, temperature, and food availability affect the clearance and growth rates of oysters, thereby influencing their ability to cycle and remove N (Newell 2004).

Bioremediation of estuaries using oysters has increasingly become a research area of interest because of the N cycling services that bivalves provide (Grizzle and Ward 2011, Carmichael et al. 2012, Beseres Pollack et al. 2013, Kellogg et al 2014). Studies examining N content of eastern oysters within estuaries on the east coast of the United State have found that oyster tissue contains between 6.5-8.6% DW nitrogen (see Table 5 for a comparison of studies). One of those studies also measured N content in shell and found that nitrogen varied between 0.17-0.26% DW depending on oyster size (Higgins et al. 2011; Table 5). While these studies have focused on the N removal capacity of eastern oysters via tissue and shell bioassimilation, none of these has focused on the Delaware Inland Bays (the Bays). The Bays, which include Rehoboth Bay, Indian River Bay, and Little Assawoman Bay are a set of shallow, eutrophied estuaries on the Mid-Atlantic coast. Although oyster aquaculture was a lucrative industry in Delaware until the 1960s, widespread disease led to its demise, and it is only now being revived (Ewart 2013). The passage of the Delaware Aquaculture Act held that "... Delaware's Inland Bays are water quality impaired and would benefit from the filtering capability of additional bivalve shellfish," drawing attention to the potential role that oysters could play in cleaning up the Bays (D. E. Legis. Assemb, 2013). Sussex County, where the Bays are located, is the number one chicken-producing county in the United States, yielding a total of 605 million chickens in 2017 (DPI 2018). Agricultural runoff from poultry farms and fertilizers (EPA 2013), combined with polluted wastewater (Ritter 1992, Volk et al. 2006) and atmospheric deposition (Scudlark et al. 2005), are the largest contributors to nitrogen entering the Bays. The county is bound by the EPA's National Pollution Discharge Elimination System (NPDES) to offset anthropogenic pollution that enters the Bays, including nitrogen. The rise of commercial aquaculture since 2013 has prompted stakeholders to explore the role that oysters could play in helping to achieve the pollution offset targets set by the NPDES.

Because N accumulation in oysters can be highly variable depending on location (Grizzle and Ward 2011, Carmichael et al. 2012) and given the recent interest in exploring oyster aquaculture as an option for pollution mitigation in the Delaware Inland Bays, N content was empirically measured at one location in each of the Bays to obtain estuary-specific information. Due to the high uncertainty surrounding the influences of oysters on denitrification and other biogeochemical processes, only N removal through tissue and shell bioassimilation was measured in this study. Using two different size classes of oysters deployed in each of the Bays, I explored whether N content in eastern oysters varied between the Bays, and whether there exists a significant difference in N accumulation rates for different size class oysters during a typical grow-out cycle.

Methods

Study location

The Delaware Inland Bays are an interconnected system of estuaries consisting of Indian River Bay, Rehoboth Bay, and Little Assawoman Bay. Located on the Mid-Atlantic coast, just north of the Maryland border, the Bays and their tributaries encompass a surface area of approximately 83 km² and have a drainage area of approximately 777 km² (Price 1998). Indian River has the largest surface area of the three with 38.3 km², followed closely by Rehoboth Bay of 37.6 km² (Ritter 1992). Little Assawoman Bay has the smallest surface area of 9.1 km² (Ritter 1992). The Bays are each very shallow (1.0-2.4 m deep) which allows for regular sediment resuspension and vertical mixing (Brown 2006). The Atlantic Ocean borders the Bays to the east, separated by an elongated baymouth barrier. The Bays experience limited tidal influence and relatively poor flushing (between 80-100 days residence time) (Price 1998). Approximately 51% of the land surrounding the Bays is used intensively for agriculture (Brown 2006). Sussex County, where the Bays are located, is the largest poultry-producing county in the United States (DPI 2017). Nutrient pollution enters the Bays from animal waste, fertilizer runoff, and contaminated groundwater in high concentrations because of these surroundings (Gutiérrez-Magness 2006, Volk et al. 2006).

Study sites

One site within each bay was chosen for oyster cultivation (Figure 1). Cages were placed at Pasture Point for Indian River Bay (N 38°34'54.8" W 75°5'15.608"), Sloan Cove for Rehoboth Bay (N 38°39'23.98" W 75°7'49.485"), and in the general vicinity of The Narrows in Little Assawoman Bay (N 38°29'46.951" W 75°3'22.729").

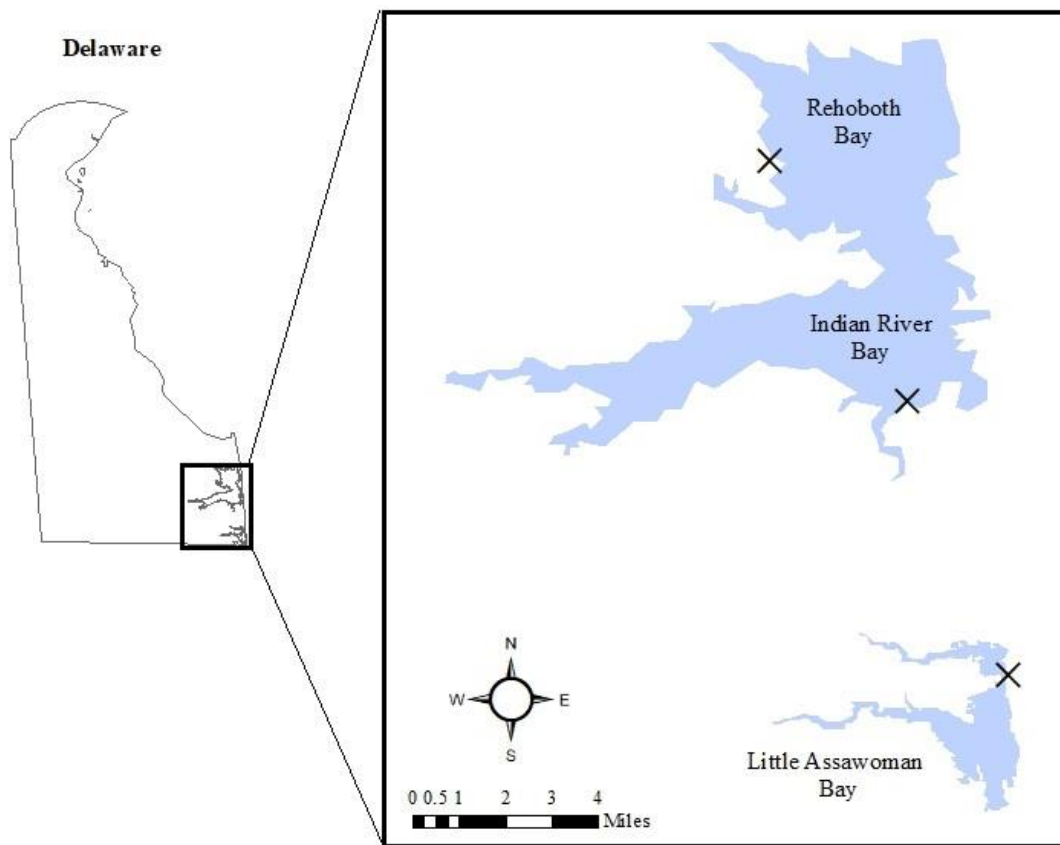


Figure 1. A map of the Delaware Inland Bays. The approximate location of the study sites is indicated with an "X."

Oyster cultivation

Diploid oysters of two different size classes, “small” (mean TL = 73.39 mm) and “medium” (mean TL = 93.43 mm), were acquired through the Delaware Inland Bays oyster gardening program in spring of 2016. The gardening program rears oysters from spat to adolescence or adulthood within the Bays (Delaware Center for the Inland Bays 2017). For reference, the typical total length for a market size oyster is approximately 76 mm TL (Newell 2004, Carmichael et al. 2012), but “jumbo” oysters can measure up to approximately 120 mm (Higgins et al. 2011). At each of the sites, replicate groups of oysters ($n = 100$) for each size class were deployed in 1.27 cm mesh Vexar™ predator exclusion bags. The bags were suspended off-bottom and supported within 5 cm mesh plastic coated wire cages that measured approximately 1 m x 1.2 m x 0.76 m (LWH or 0.85 m³) in size. The cages were fixed in shallow water about 1 m in depth. Two cages, one for each size class of oysters, were secured at each location. Oysters were cultivated from April until November 2016. The growth period of April to November was chosen to represent a typical oyster grow-out season; oyster spat are planted in the spring (April-May) and harvested in the fall (October-November) approximately 2 years later. The fastest growth occurs in the

summer months when the temperatures are highest and food availability is greatest. During the grow-out process, gear was maintained to maximize water exchange and minimize oyster mortality.

Data collection

At the start of the cultivation period, oysters (n = 10 per size class, per bay) were randomly selected for biological measurements and N content analysis of the tissue and shell. These measurements were used to establish initial nutrient contents against which final nutrient contents could be compared. The oysters grew from April to November 2016, after which measurements were made on randomly selected oysters (n = 35 per size class, per bay) for statistical analysis to estimate the N assimilation services provided by the oysters (Table 1). The measurements taken prior to the cultivation period are referred to here as “initial” measurements, and those collected after the experiment are referred to as “end” measurements.

Table 1. Summary table of oyster sample size. Treatment and total n represent the number of oysters per size class, per bay.

	Sample	Total n
Sampling Phase 1 (“initial”)	10	100
Sampling Phase 2 (“end”)	35	90*

*This quantity does not include incidental mortality that may have occurred during the experiment; such mortalities were not recorded.

The morphometric parameters recorded for each oyster sampled included shell height, width, length, total wet weight, shell wet weight, tissue wet weight, shell dry weight and tissue dry weight. After shell and tissue were separated, dehydrated, and weighed, samples of the dried tissue and shell for each individual were sent to the Stable Isotope Geosciences Facility at Texas A&M for N content analysis. The samples were ground to a fine powder using a mortar and pestle and then assayed for nitrogen by combustion using a CHN analyzer. N content was recorded as % DW (dry weight) and in milligrams for the tissue sample, and in milligrams only for the shell sample. The nutrient content analysis was completed in November 2017.

Data transformation and statistical analysis

Before performing the statistical analysis, all data were first transformed to achieve uniformity in the data set. N content of shell expressed in % DW was calculated for each individual by dividing the total mass of N (mg) in the sample by the mass of the sample (mg) and converting those values to a percent:

$$\% DW_{\text{shell}} = [\text{total mass N (mg)} / \text{mass of sample (mg)}] * 100$$

The total N content of the tissue and shell was then calculated by multiplying the % DW by the total weight of the dehydrated tissue and shell, respectively. Two observations, one from Indian River Bay and one from Rehoboth Bay, were removed from the data set because they represented unrealistic values. The lab confirmed that these observations were measured incorrectly and should not be included in the analysis. Both observations classified as end measurements from the small size class.

A statistical analysis was performed using R Version 3.5.3 (R Development Core Team 2018) to compare N accumulation in oyster tissue and shell between sites and size classes. Observations were excluded from the analysis if their associated morphological or N content measurements were missing. A one-way ANOVA was used to determine whether there was a significant difference in N content of oysters between aquaculture sites. Only the end measurements were included in this part of the analysis (n = 35 per bay) to eliminate the possibility of the initial oyster measurements obscuring any possible difference in N accumulation, because the initial oyster N does not vary across bays. To ensure that the initial measurements were not statistically different by bay, and thereby justify their exclusion from this part of the analysis, a preliminary one-way ANOVA was performed on only the initial measurements. This preliminary test showed no significant difference in N content between bays for the oysters at time zero. I therefore concluded that using only the end measurements in the ANOVA comparing N accumulation rates between bays was a reasonable estimate of N accumulation across bays.

Welch two sample t-tests were then used to determine whether N assimilation into tissue and shell of oysters differed significantly among size class. Oyster data were pooled across bays so that only size classes, not bays, were compared. To calculate the rate of N accumulation for each size class, the mean N (mg) of initial oysters was subtracted from each of the N end measurements (mg) to obtain a set of N accumulation rates for each size class (mg N/year). For example:

If TAR_{ij} = total N accumulation rate for oysters of size class i and size class j,

And N_{ij} = end nitrogen content for oyster $i = 1, \dots, 35$ from size class j in {S, L},

And X_k = initial nitrogen content from oyster $k = 1, \dots, 10$

Then $TAR_{ij} = N_{ij} - \bar{x}$, so that $\overline{TAR_j}$ = the mean total accumulation rate for each size class.

The set of N accumulation rates for small size class oysters was then compared to that of medium size class oysters using a t-test. Separate tests were completed for N accumulation in tissue and shell.

Results

Morphometric analysis

After the cultivation period, small size class oysters ($n = 69$) exhibited mean TL of 76.83 mm, mean tissue DW of 1.49 g, mean shell DW of 32.02 g, and total DW of 33.51 g, representing an approximate 5% increase in TL and a 26% increase in total DW. Small oysters gained 0.56 g in tissue and 6.42 g in shell DW, a 60% and 25% weight increase, respectively. Medium size class oysters ($n = 105$) exhibited mean TL of 93.01 mm, mean tissue DW of 2.45 g, mean shell DW of 51.39 g, and total DW of 53.83 g. They gained 1.05 g in tissue and 12.59 g in shell DW, a 75% and 32% weight increase, respectively. Overall, medium oysters did not increase in total length but showed an approximate 34% increase in total DW (Table 2).

Table 2. Summary table of oyster morphological features and N content by size.

	Size	n	Morphometrics		Nitrogen	
			TL (mm) \pm SD	DW (g) \pm SD	% DW \pm SD	Mean (g) \pm SD
Shell	initial small	29	73.58 \pm 7.84	25.6 \pm 5.89	0.57 \pm 0.27	0.15 \pm 0.09
	end small	69	76.83 \pm 10.82	32.02 \pm 12.14	1.02 \pm 0.47	0.34 \pm 0.26
	initial med	30	93.43 \pm 7.3	38.8 \pm 8.34	0.56 \pm 0.24	0.22 \pm 0.11
	end med	105	93.01 \pm 8.8	51.39 \pm 13.95	0.85 \pm 0.29	0.44 \pm 0.21
Tissue	initial small	29	73.58 \pm 7.84	0.93 \pm 0.26	8.3 \pm 0.65	0.077 \pm 0.022
	end small	69	76.83 \pm 10.82	1.49 \pm 0.56	8.1 \pm 1.1	0.12 \pm 0.042
	initial med	30	93.43 \pm 7.3	1.4 \pm 0.33	8.1 \pm 0.76	0.11 \pm 0.025
	end med	105	93.01 \pm 8.8	2.45 \pm 1.07	7.43 \pm 0.94	0.18 \pm 0.068
Total	initial small	29	73.58 \pm 7.84	26.53 \pm 6.02	0.84 \pm 0.28	0.22 \pm 0.1
	end small	69	76.83 \pm 10.82	33.51 \pm 12.35	1.35 \pm 0.44	0.46 \pm 0.27
	initial med	30	93.43 \pm 7.3	40.2 \pm 8.53	0.82 \pm 0.24	0.33 \pm 0.13
	end med	105	93.01 \pm 8.8	53.83 \pm 14.77	1.14 \pm 0.3	0.61 \pm 0.25

Note: End measurements for small size class oysters from Indian River ($n = 34$) were not included in the morphometric analysis because length, height and width were not recorded for this group (Table 2). Of these 34 observations, 16 individuals were also missing measurements of N content in the shell and were therefore not included in the N content analysis (Table 3).

Nitrogen accumulation

After the cultivation period, N content ranged from 0.85 to 1.02% DW in oyster shell and from 7.43% to 8.1% DW in oyster tissue (end measurements, Table 2). The proportion of N content in tissue and shell, measured as % DW, remained relatively constant between the initial and end measurements. Mean total N (g) was greatest in Indian River Bay and lowest in Rehoboth Bay (Table 3). Individual oysters contained

mean total N of 0.56, 0.46 and 0.43 g in Indian River (n = 73), Rehoboth Bay (n = 90), and Little Assawoman Bay (n = 88), respectively.

Table 3. Summary table of N content by bay.

Component	Bay	N	Nitrogen	
			%DW±SD	Mean (g)±SD
Shell	IR	73	0.73±0.29	0.39±0.25
	LAB	90	0.91±0.32	0.33±0.17
	RB	88	0.81±0.47	0.32±0.25
Tissue	IR	89	7.5±0.88	0.17±0.07
	LAB	90	7.7±1.1	0.14±0.052
	RB	88	8.3±0.76	0.11±0.038
Total	IR	73	1±0.29	0.56±0.3
	LAB	90	1.2±0.33	0.46±0.21
	RB	88	1.1±0.46	0.43±0.26

On average, oysters in the small size class accumulated approximately 0.19 g of nitrogen in the shell and 0.04 g N in the tissue, while medium size class oysters accumulated approximately 0.22 and 0.07 g N in the shell and tissue, respectively (Table 2). These numbers represent an approximate 127% and 52% increase in the shell and tissue for small class oysters, and 100% and 64% increase in the shell and tissue for medium class oysters, respectively.

ANOVA

One-way ANOVA analysis revealed that mean total N (g) content varied significantly between bays for tissue, but not for shell (Table 4). Nitrogen content in tissue was significant at the 0% level ($F= 36.558$ and $p= 2.64e-14$). Oysters from Indian River had the greatest N content in both tissue and shell, but only N content in tissue was significantly different from that of Rehoboth and Little Assawoman Bays.

Table 4. ANOVA results comparing N content, broken into tissue and shell, by bay.

Component	term	df	sumsq	meansq	statistic	p.value
tissue	bay	2	0.20	0.10	36.56	0.00
	Residuals	205	0.57	0.00	NA	NA
shell	bay	2	0.42	0.21	4.21	0.02
	Residuals	189	9.47	0.05	NA	NA
total	bay	2	1.25	0.63	10.27	0.00
	Residuals	189	11.55	0.06	NA	NA

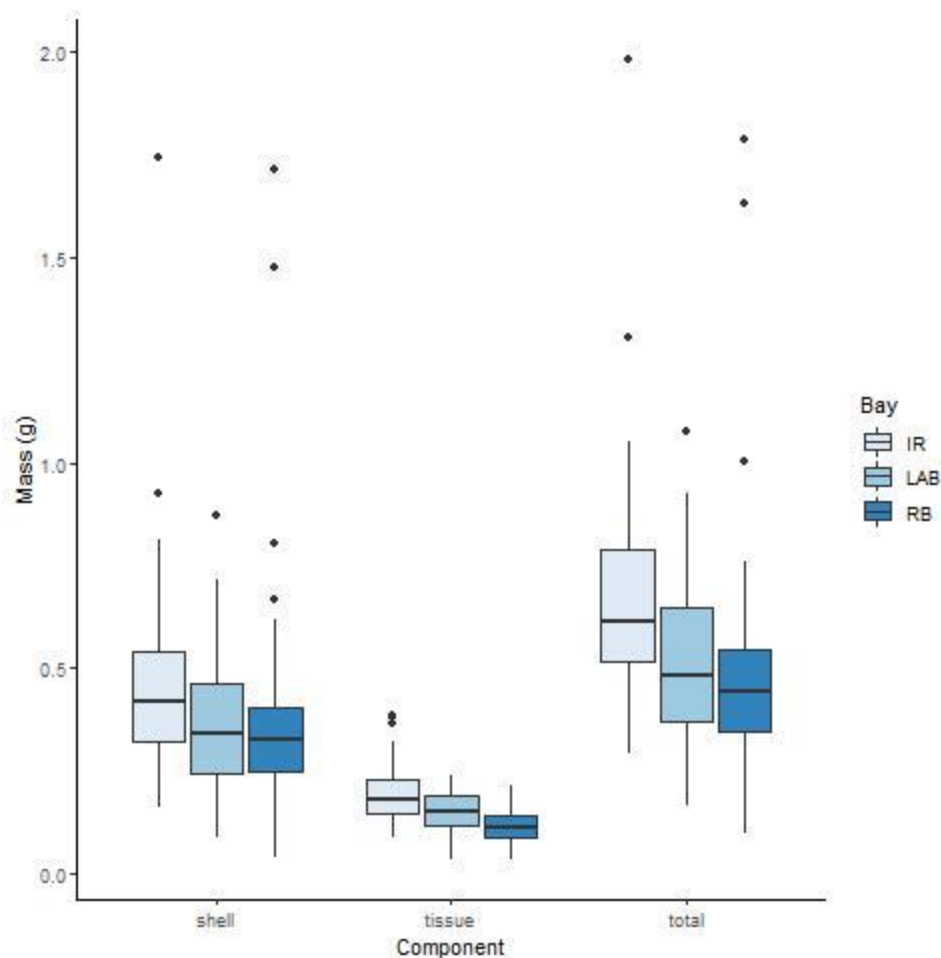


Figure 1. Nitrogen content (g) across bays. The middle line represents the median, the lower and upper hinge represent the 25% and 75% quartiles, respectively, and the whiskers extend for $1.5 \times \text{IQR}$ (or the inter-quartile range) (R Development Core Team 2018).

Welch two sample t-test

A Welch two sample t-test revealed that there was no significant difference in N accumulation rate ($\text{g} \cdot \text{year}^{-1}$) within tissue between the different size class oysters, pooled across all bays ($t = -1.8882$, $df = 170.5$, $p\text{-value} = 0.0607$), though it should be noted that the p-value is bordering significance. The mean accumulation rate in tissue of small and medium oysters was $.050$ and $.065 \text{ g} \cdot \text{year}^{-1}$, respectively (Figure 3). A second t-test revealed that there was no significant difference in N accumulation rate within shell between the different size class oysters, pooled across bays ($t = -0.77816$, $df = 170.5$, $p\text{-value} = 0.4376$). The mean accumulation rate in shell of small and medium oysters was 0.19 and $.22 \text{ g} \cdot \text{year}^{-1}$, respectively.

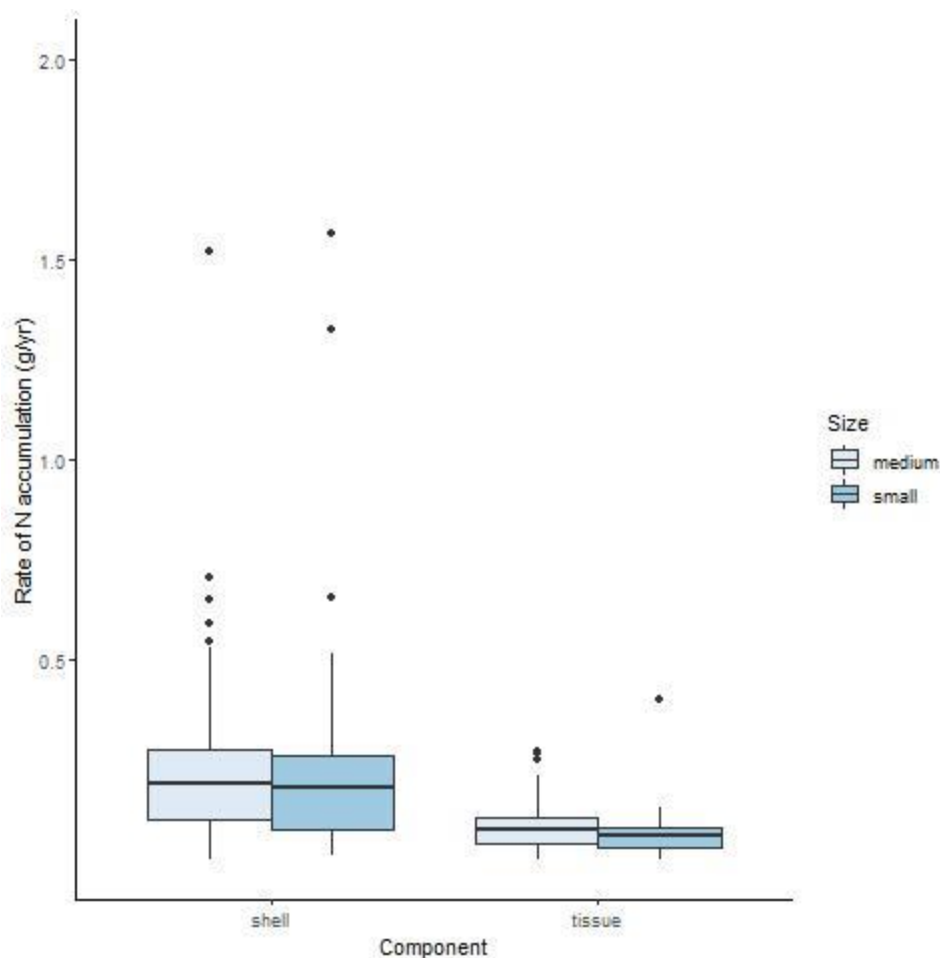


Figure 2. Rate of N accumulation in shell and tissue. The middle line represents the median, the lower and upper hinge represent the 25% and 75% quartiles, respectively, and the whiskers extend for 1.5*IQR (or the inter-quartile range) (R Development Core Team 2018).

Discussion

Morphometric features

Oysters exhibited an increase in total mass over the cultivation period, indicating that nutrient bioaccumulation occurred (Table 2). Total dry weights observed in this study are similar to those reported in Higgins et al. (2011) and are three to five times less than wild oysters (Newell et al. 2005). Oysters grown in cages weigh less than wild oysters because they lack the need for a strong shell for protection, resulting in a thinner, lighter shell (Higgins et al. 2011). Oyster tissue increased by approximately 1 g, which is typical for one year of growth (Higgins et al. 2011, Carmichael et al. 2012). Despite the increase in total mass, oysters exhibited little to no increase in total length over the grow-out stage (Table 2). While I expected to see greater increases in total length, the observed rates are not anomalous compared to previous reports. A review of eastern oyster growth rates reveals that increases in total length can be highly variable: for oysters approximately the same size as those used this study, growth varied from 1

mm to 22.6 mm over a similar time period (Krauter et al. 2007; Table 5). Additionally, Krauter et al. (2007) noted that growth seems to steadily decline starting around the time that oysters reach market size (~76 mm), or after 1-2 years of growth (see also Carmichael et al. 2012). The oysters used in this study were close to, or had already reached, market size before they were planted, which may explain why they experienced limited growth in total length. Other factors that slow growth rates include limited food supply, extreme temperatures, and low salinity (Newell et al. 2005, Krauter et al. 2007, Wang et al. 2008). Because the Bays are nutrient-rich estuaries (Delaware Inland Bays Estuary Program 1991, DNREC 2008), I do not suspect that food supply was limited or that lack of access to food would have slowed growth. Because I do not have temperature or salinity data for these sites, I am unable to estimate the potential effects of temperature or salinity on growth. Low salinity could have contributed to slow growth rates if freshwater inputs were large, but without analyzing data on freshwater inputs, I have no evidence to support that conclusion. While the oysters did not grow much in total length, their respective tissue and dry weights at the end of the study are consistent with other reported values (Table 5).

Table 5. Comparison of studies examining N content in estuaries on the east coast of the United States.

Location	Starting length (mm)	End length (mm)	Cultivation period	Tissue DW (g)	Shell DW (g)	N tissue (g)	N tissue (%DW)	N shell (g)	N shell (%DW)
Great Bay estuaries, NH ¹	10-15	35.7	3 months	0.06	n/d	0.01*	6.52	n/d	n/d
Great Bay estuaries, NH ¹	30-40	55.6	3 months	0.24	n/d	0.07*	7.86	n/d	n/d
Chesapeake Bay ²	n/d	76	n/d	1.00	150	0.07	7	0.45	0.3**
Chesapeake Bay ³	12	43.6	up to 2 yrs	0.20	4.8	0.016	8.15	0.009	0.18
Chesapeake Bay ³	12	64.8	up to 2 yrs	0.80	24.3	0.064	8.06	0.048	0.19
Chesapeake Bay ³	12	85.5	up to 2 yrs	1.58	37.6	0.113	7.28	0.062	0.17
Chesapeake Bay ³	12	117.8	up to 2 yrs	3.00	71.9	0.216	7.37	0.177	0.26
Cape Cod, MA ⁴	8.2	46	112 days	n/d	n/d	0.2-0.4*	8.6	n/d	n/d
Cape Cod, MA ⁴	8.2	76.2	1.8 years	n/d	n/d	0.3-0.5*	8.6	n/d	n/d
Delaware Inland Bays	73.58	76.83	8 months	1.49	32.02	0.12*	8.1	0.34	1.02
Delaware Inland Bays	93.43	93.01	8 months	2.45	51.39	0.18*	7.43	0.44	0.85

*indicates a significant difference found in N tissue (%DW) among sites

**value used from literature; not measured in the study

¹Grizzle and Ward (2011), ²Newell et al. (2005), ³Higgins et al. (2011), ⁴Carmichael et al (2012)

N content in tissue and shell

The N content within oyster tissue at the end of the cultivation period varied between 7.43-8.1% DW, which is consistent with previous estimates (Table 5). N content measured as % DW is higher in the tissue than in the shell, which was expected given that oysters assimilate more N as a function of dry

weight in their tissue than their shell (Newell 2004). When measured in grams, however, N content is up to almost four times lower in the tissue than in the shell, which is inconsistent with previous studies that report that N content varies between 0.1-0.3% DW in the shell (Newell et al. 2004; Higgins et al. 2011; see Table 5). In the Delaware Inland Bays, N content in shell averaged 0.85 and 1.02% DW for small and medium size class oysters, respectively (Table 2), which is up to a magnitude greater than the values reported in Higgins et al. (2011). One explanation could be that there are several outliers in this study, indicated by the points beyond the whiskers in Figure 2, whose measurements could have slightly inflated the mean total N in the shell (Figure 2). Another explanation could be that N assimilation in shell is more variable than previously thought, which would provide further evidence that N removal via bioaccumulation depends on specific estuary conditions.

Spatial and temporal patterns of N content in the Delaware Inland Bays

N assimilation did not differ significantly between size classes of oysters (Figure 3); however, it did vary spatially (Figure 2). While no previous studies have compared N content between different size classes of oysters, other reports have found N content to vary between 6.5-8.6% DW in tissue despite the size of the oyster (Newell et al. 2004, Higgins et al. 2011, Carmichael et al. 2012, Kellogg et al. 2014), which is further supported by this analysis. Only one other study on the east coast of the US has empirically measured N content in oyster shell (Higgins et al. 2011), so it is difficult to make a generalization about the variation in N content in shell of *C virginica*.

A spatial difference in N accumulation in tissue was observed, with oysters grown at Indian River having the greatest N content followed by Little Assawoman Bay and Rehoboth Bay (Figure 2). Like growth, N accumulation can vary greatly between sites, even sites within an estuary, because of factors including temperature, N input, salinity, hydrology, bivalve density, food availability and dissolved oxygen levels (Pietros and Rice 2003, Kraeuter et al. 2007, Wang et al. 2008, Grizzle and Ward 2011, Luckenbach 2013, Smyth et al. 2018). The spatial difference in N content observed in this study suggests that one or more of these factors affected N content in oyster tissue. Hydrological data and water quality reports reveal that N input is greatest in Indian River Bay (Delaware Center for the Inland Bays 2016), which is also the location that showed the greatest N content in oyster tissue and shell (Figure 2). However, Rehoboth Bay has the second highest N input, followed by Little Assawoman Bay, which does not match the sequential order of N content in oysters observed in this study. These relationships show that N availability is not the only predictor of N accumulation in oysters within the Delaware Inland Bays. Other factors such as salinity are likely associated with variable N content in oysters. Salinity follows a longitudinal gradient in the Bays, such that westernmost points have a lower salinity due to the proximity

to freshwater flushing from rivers, and easternmost points have a greater salinity because they are more tidally flushed (Xu et al. 2006, Price 1998). Based on this relationship, and in the absence of site-specific salinity data, I would expect the Rehoboth site to have the greatest freshwater input and therefore the lowest salinity, followed by the Indian River and Little Assawoman sites, respectively (Figure 1). If this were the case, I would then expect oysters from Rehoboth to have lower N content than oysters from the other sites. The data show that Rehoboth did indeed have the lowest N content of the three bays, so low salinity could offer a possible explanation.

The potential of annual N removal in the Bays

Using predictive models for nutrient content based on shell length, Higgins et al. (2011) estimated that 10^6 oysters, size 76 mm TL, would remove 132 kg N·year⁻¹ in the Chesapeake Bay via N bioaccumulation in shell and tissue. Using the mean accumulation rate of similar size oysters (TL = 76.83 mm, mean accumulation rate = 0.24 g·year⁻¹) from this study, the same volume of oysters would remove 240 kg N·year⁻¹ in the Delaware Inland Bays. The Total Maximum Daily Load (TMDL) of nitrogen into the smallest bay, Little Assawoman, is 269.66 kg·day⁻¹, close to what it would take one million oysters to remove in an entire year (DNREC 2008). However, research in the Delaware Inland Bays has estimated that up to 750,000 oysters may be harvested per acre, per year (Delaware Center for Inland Bays 2013). In 2011, 160-acres of leased aquaculture areas were in operation in the Bays. If those areas operated under full capacity, and assuming 0% oyster mortality, an estimated 55,200 kg N·year⁻¹ could be removed from the Bays via nutrient assimilation, or about 50% of the total N allowed to enter Little Assawoman Bay under the current TMDL regulations. This rough calculation indicates that not all N entering the Delaware Inland Bays could be extracted using oysters alone. Increasing the volume of oyster cultivation may seem like a viable option to achieve higher N removal, but an important consideration is spatial limitation of the environment. The Delaware Inland Bays comprise a relatively small area where multi-use activities often compete for space, and therefore may not be able to support high oyster density. Greater oyster density has other ecological effects as well, including decreased dissolved oxygen levels which can affect other organisms in the ecosystem (Dame 2012).

My rough estimates support other work that has shown that bioremediation using bivalve aquaculture would only be possible in the least-impaired estuaries because of the relatively low N accumulation and limited space for oyster cultivation (Carmichael et al. 2012). Estuaries in greatest need of remediation likely do not have the resources, such as space, to support enough oysters to achieve complete, or even substantial, N removal. Other studies have drawn similar conclusions, reporting less than 20% of total N loads to be removed via bivalve remediation (Coen et al. 2007, Carmichael et al. 2012). While oysters are

unlikely to be the single solution to N removal, they could be considered as part of a larger nitrogen removal plan.

N removal via bioassimilation vs. other N removal pathways

This study considered N removal from the water column via bioaccumulation only and did not include added potential removal from other biogeochemical processes. N removal via biogeochemical processes such as biodeposition of feces and pseudofeces and denitrification is reportedly slower during the initial grow-out stage but becomes more important after oysters reach market size (Carmichael et al. 2012, Humphries et al. 2016). These studies suggest that oysters left in the estuary beyond the time when they reach harvestable size could therefore achieve slightly higher N removal by remaining in the estuary beyond one to two years. However, maintaining oysters beyond market size in an aquaculture operation is not the most cost-effective decision for the growers unless they are additionally compensated for the nutrient removal services provided by oysters. This is because the growers will incur additional costs while cultivating the oysters while also being compensated less for the oysters once harvested, since smaller oysters (~76 mm) are more desirable and therefore have a greater market value.

Implications for policymakers

If policymakers in Sussex County are interested in using commercial bivalve aquaculture as part of the nutrient trading program to help mitigate N input into the Delaware Inland Bays, the following points should be considered, based on the findings of this study:

1. Improved water quality resulting from the practice of oyster aquaculture represents a positive economic externality, and the nutrient removal services that oysters provide at no cost can be considered a public good (Flood, in press). If a nutrient trading program aimed to implement additional offsets than those provided by the status quo, growers would need to be paid an additional fee to account for the nutrient removal services, since growers currently receive compensation for only the commercial sale of oysters. However, if no additional offsets are achieved under the nutrient trading program compared to the status quo, then growers would not need to receive additional compensation because they would not be providing additional nutrient offsets.
2. The ecosystem service provision of N assimilation is location-dependent within the Delaware Inland Bays. A nutrient trading program would therefore need to consider that aquaculture operations in different areas of the estuary will achieve significantly different N removal. This variation must be accounted for when compensating growers for aquaculture-mediated N removal. For example, if a program assumed that N accumulation was equal to the mean

accumulation rate across all sites, they would underestimate N removal in Indian River Bay by roughly $0.08 \text{ g}\cdot\text{year}^{-1}\cdot\text{oyster}^{-1}$, and overestimate N removal in Rehoboth and Little Assawoman Bays by roughly 0.05 and $0.02 \text{ g}\cdot\text{year}^{-1}\cdot\text{oyster}^{-1}$, respectively. With 750,000 oysters harvested per acre, this discrepancy could lead to an underestimate of N removal in Indian River Bay of $60 \text{ kg}\cdot\text{year}^{-1}\cdot\text{acre}^{-1}$ and an overestimation of N removal in Rehoboth and Little Assawoman Bays by 37.5 and $15 \text{ kg}\cdot\text{year}^{-1}\cdot\text{acre}^{-1}$, respectively.

3. Accounting for N removal via other biogeochemical pathways in a nutrient trading program might be an attractive option because N removal may be slightly higher when these processes are considered; however, these processes are complicated and have yet to be reliably quantified. While literature largely agrees that denitrification is highly variable according to location, season, habitat type and oyster density, it disagrees on whether these processes lead to net removal, regeneration or recycling of nitrogen (Newell 2002, Pietros and Rice 2003, Humphries et al. 2006, Luckenbach 2013, Smyth et al. 2015, Smyth et al. 2018). Presently, the most reliable quantification of N removal from the water column is through accumulation in oyster tissue and shell. Therefore, bioassimilation should continue to be the only pathway considered when quantifying N removal for use in nutrient trading programs.

Challenges with the analysis

Observations with missing measurements were removed which ultimately decreased sample sizes used in the analysis. Notably, 36 of 105 “end” measurements for small oysters and 17 of 90 shell measurements from Indian River were not included. Secondly, because of permitting restrictions surrounding spat acquisition, the oysters in this study had nearly reached adulthood, and therefore already contained notable quantities of N in the tissue and shell (Table 2). There is a possibility that larger initial size oysters obscured the respective rates of N assimilation in tissue and shell over the course of this study (for example, see Kraeuter et al. 2007 and Carmichael et al. 2012).

Conclusion

Bivalve-mediated N removal from estuarine systems via biological assimilation depends on estuary and location-specific conditions including salinity, food supply, and temperature. This study aimed to understand N bioaccumulation in the Delaware Inland Bays across space and across size class. The results agree with previous studies that N stored in oyster tissue varies between 6.5-8.6% DW. However, the observed N content in the shell offers the possibility that oysters are capable of storing up to an order of magnitude more nitrogen in their shell than previously believed. Although there was no significant difference between the N accumulation rates between size classes, future studies comparing accumulation

rates of smaller oysters (~10 mm) to larger oysters (~76 mm) could have different, and interesting, results given oyster growth rates.

The more fully we understand the multiple pathways by which the presence of oysters influences N cycling, the better able we are to quantify the value of the nutrient removal services that oysters provide. I was able to successfully quantify the biological N content of eastern oysters grown in the Delaware Inland Bays, but more empirical studies are needed to understand net N removal, which includes accounting for biogeochemical processes such as denitrification. Because N cycling can be highly variable, more research on the scale of these processes, specifically within the Delaware Inland Bays, is needed to develop a comprehensive nutrient trading program. Presently, our best understanding of N removal is that which occurs through physical harvest after N bioassimilation in the tissue and shell of oysters. Until N cycling can be reliably quantified at the sediment and ecosystem levels, managers should consider only bioassimilation measures of N in the development of nutrient trading programs with respect to nitrogen.

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